AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

- 1.-32. (Canceled).
- 33. (Currently Amended) A reagent for use in detecting an analyte, comprising a fluorescent energy donor and an energy acceptor, the energy donor and the energy acceptor being such that when they are sufficiently close to one another energy is non-radiatively transferred from the energy donor following excitation thereof to the energy acceptor quenching fluorescence of the energy donor, wherein the energy acceptor is of the formula:

wherein:

 R^1 , R^2 and R^3 are each independently H, electron donating substituents, or electron withdrawing substituents or R^3 is attached to a linker structure, provided that at least two of R^1 , R^2 and R^3 are electron donating groups;

R⁴, R⁵, R⁶, R⁷, R⁸ and R⁹ are each independently H, halogen, alkyl, aryl, O-alkyl, S-alkyl and R¹⁰, R¹¹, R¹², R¹³, R¹⁴ and R¹⁵ are each independently hydrogen, O-alkyl, S-alkyl, alkyl, or one or more pairs of groups R¹ and R⁴ and/or R¹ and R⁵ and/or R² and R⁶ and/or R² and R⁷ and/or R³ and R⁸ and/or R³ and R⁹ and/or R⁴ and R¹⁰ and/or R⁵ and R¹¹ and/or R⁶ and R¹² and/or R⁷ and R¹³ and/or R⁸ and R¹⁴ and/or R⁹ and R¹⁵ is a bridging group consisting of aryl, alkylene, O-

alkylene, S-alkylene or N-alkylene optionally substituted with one or more of SO₃⁻, PO₃²-, OH, O-alkyl, SH, S-alkyl, COOH, COO⁻, ester, amide, halogen, SO-alkyl, SO₂-alkyl, SO₂NH₂, SO₂NH-alkyl, SO₂N-dialkyl, SO₃-alkyl, CN, secondary amine or tertiary amine, provided that not all of R¹⁰, R¹¹, R¹², R¹³, R¹⁴ and R¹⁵ are hydrogen and at least one of R¹⁰, R¹¹, R¹², R¹³, R¹⁴ and R¹⁵ is O-alkyl;

and wherein the distance between the energy donor and the energy acceptor of the reagent is capable of modulation by a suitable analyte to be detected.

- 34. (Previously Presented) A reagent as claimed in claim 33, wherein the energy donor and energy acceptor are linked together by a covalent linkage.
- 35. (Previously Presented) A reagent as claimed in claim 34, wherein the covalent linkage between the energy donor and energy acceptor is cleavable to increase the distance between the energy donor and the energy acceptor of the reagent.
- 36. (Previously Presented) A reagent as claimed in claim 34, wherein the energy donor and energy acceptor are linked via a polynucleotide sequence or a polynucleotide analogue sequence or a polypeptide sequence, the sequence having a conformation which is capable of modulation by a suitable analyte to be detected so as to modulate the distance between the energy donor and the energy acceptor of the reagent.
- 37. (Previously Presented) A reagent as claimed in claim 33, wherein the energy donor and energy acceptor are linked together by non-covalent binding.
- 38. (Previously Presented) A reagent as claimed in claim 37 wherein the non-covalent binding exists between an analyte binding agent linked to one of the energy donor and the energy acceptor and an analyte analogue linked to the other of the energy donor and the energy acceptor, the non-covalent binding being disruptable by a suitable analyte so as to increase the distance between the energy donor and the energy acceptor of the reagent.
- 39. (Previously Presented) A reagent as claimed in claim 38, wherein the analyte binding agent is a lectin.
- 40. (Previously Presented) A reagent as claimed in claim 38, wherein the analyte analogue is a glucose analogue.
- 41. (Previously Presented) A reagent as claimed in claim 40, wherein the analyte analogue is dextran.

- 42. (Previously Presented) A reagent as claimed in claim 33, wherein the energy donor and the energy acceptor are not linked in the absence of analyte.
- 43. (Previously Presented) A reagent as claimed in claim 33, wherein a linker structure is attached to the energy acceptor at R³, or where a bridging group is present optionally the linker structure is attached to the energy acceptor at the bridging group.
- 44. (Previously Presented) A reagent as claimed in claim 33, wherein the electron donating substituents are selected from amino, primary amine, secondary amine, O-alkyl, alkyl, S-alkyl, amide, ester, OH and SH.
- 45. (Previously Presented) A reagent as claimed in claim 44, wherein one or more of R¹ to R³ is dimethylamino, diethylamino or methylethylamino, optionally substituted with one or more of SO₃⁻, PO₃²⁻, OH, O-alkyl, SH, S-alkyl, COOH, COO⁻, ester, amide, halogen, SO-alkyl, SO₂-alkyl, SO₂NH-alkyl, SO₂NH-alkyl, SO₂N-dialkyl, SO₃-alkyl, CN, secondary amine or tertiary amine.
- 46. (Previously Presented) A reagent as claimed in claim 33, wherein an electron withdrawing substituent is present, and the electron withdrawing substituent is selected from NO, NO₂, CN, COOH, ester, COO⁻, amide, CHO, keto, SO-alkyl, SO₂-alkyl, SO₂NH-alkyl, SO₂N-dialkyl, and SO₃-alkyl.
- 47. (Previously Presented) A reagent as claimed in claim 33, wherein at least one of R^{10} , R^{11} , R^{12} , R^{13} , R^{14} and R^{15} is O-alkyl.
- 48. (Previously Presented) A reagent as claimed in claim 33, wherein one or more pairs of groups R⁴ and R¹⁰ and/or R⁵ and R¹¹ and/or R⁶ and R¹² and/or R⁷ and R¹³ and/or R⁸ and R¹⁴ and/or R⁹ and R¹⁵ is a bridging group consisting of alkylene, O-alkylene, S-alkylene or N-alkylene optionally substituted with one or more of SO₃⁻, PO₃²⁻, OH, O-alkyl, SH, S-alkyl, COOH, COO⁻, ester, amide, halogen, SO-alkyl, SO₂-alkyl, SO₂NH₂, SO₂NH-alkyl, SO₂N-dialkyl, SO₃-alkyl, CN, secondary amine or tertiary amine.
- 49. (Previously Presented) A reagent as claimed in claim 33, wherein R¹⁰ to R¹⁵ are each Omethyl or O-ethyl.
- 50. (Previously Presented) A reagent as claimed in claim 33, further comprising one or more counterions selected from halide, BF₄, PF₆, NO₃, carboxylate, ClO₄, Li₃, Na₃, K₃, Mg₃²⁺ and Zn²⁺.
- 51. (Previously Presented) A reagent as claimed in claim 33, wherein a linker structure is

present, and is formed by reaction of a linker element selected from an active ester, an isothiocyanate, an acid chloride, an aldehyde, an azide, an α -halogenated ketone and an amine with a reaction partner.

- 52. (Previously Presented) A reagent as claimed in claim 51, wherein the reaction partner is selected from a polysaccharide, a polynucleotide and a protein.
- 53. (Previously Presented) A reagent as claimed in claim 51, wherein the linker element is an active ester, and is selected from succinimidyl and pentafluorophenyl active esters.
- 54. (Currently Amended) A reagent as claimed in claim 33, wherein the energy donor is Alexa Fluor-594TMa dye that absorbs at 594 nm and fluoresces at 620 nm.
- 55. (Withdrawn) A dye compound having the general formula:

wherein:

R⁴, R⁵, R⁶, R⁷, R⁸ and R⁹ are each independently H, halogen, alkyl, aryl, O-alkyl or S-alkyl and R¹⁰, R¹¹, R¹², R¹³, R¹⁴ and R¹⁵ are each independently hydrogen, O-alkyl, S-alkyl, or alkyl, or one or more pairs of groups R²⁰ and R⁴ and/or R²⁰ and R⁵ and/or R⁴ and R¹⁰ and/or R⁵

and R¹¹ and/or R⁶ and R¹² and/or R⁷ and R¹³ and/or R⁸ and R¹⁴ and/or R⁹ and R¹⁵ is a bridging group consisting of aryl, alkylene, O-alkylene, S-alkylene or N-alkylene optionally substituted with one or more of SO₃⁻, PO₃²-, OH, O-alkyl, SH, S-alkyl, COOH, COO⁻, ester, amide, halogen, SO-alkyl, SO₂-alkyl, SO₂NH₂, SO₂NH-alkyl, SO₂N-dialkyl, SO₃-alkyl, CN, secondary amine or tertiary amine, provided that not all of R¹⁰, R¹¹, R¹², R¹³, R¹⁴ and R¹⁵ are hydrogen;

R¹⁶, R¹⁷, R¹⁸ and R¹⁹ are each independently H, alkyl or aryl, or one or more of R¹⁶ and R¹⁷ or R¹⁸ and R¹⁹ is alkylene, optionally substituted with one or more of SO₃⁻, PO₃²⁻, OH, O-alkyl, SH, S-alkyl, COOH, COO⁻, ester, amide, halogen, SO-alkyl, SO₂-alkyl, SO₂NH₂, SO₂NH-alkyl, SO₂N-dialkyl, SO₃-alkyl, CN, secondary amine or tertiary amine;

or one or more of pairs of groups R⁶ and R¹⁶, R⁷ and R¹⁷, R⁸ and R¹⁸ and R⁹ and R¹⁹ is alkylene, O-alkylene, S-alkylene or N-alkylene optionally substituted with one or more of SO₃⁻, PO₃²⁻, OH, O-alkyl, SH, S-alkyl, COOH, COO⁻, ester, amide, halogen, SO-alkyl, SO₂-alkyl, SO₂NH₂, SO₂NH-alkyl, SO₂N-dialkyl, SO₃-alkyl, CN, secondary amine or tertiary amine and

 R^{20} is a linker element selected from an active ester, an isothiocyanate, an acid chloride, an α -halogenated ketone and an azide.

- 56. (Withdrawn) A dye compound as claimed in Claim 55, wherein at least one of R^{10} , R^{11} , R^{12} , R^{13} , R^{14} and R^{15} is alkyl.
- 57. (Withdrawn) A dye compound as claimed in Claim 56, wherein one or more pairs of groups R⁴ and R¹⁰ and/or R⁵ and R¹¹ and/or R⁶ and R¹² and/or R⁷ and R¹³ and/or R⁸ and R¹⁴ and/or R⁹ and R¹⁵ is a bridging group consisting of alkylene, O-alkylene, S-alkylene or N-alkylene optionally substituted with one or more of SO₃, PO₃²⁻, OH, O-alkyl, SH, S-alkyl,

COOH, COO, ester, amide, halogen, SO-alkyl, SO₂NH₂, SO₂NH-alkyl, SO₂N-dialkyl, SO₃-alkyl, CN, secondary amine or tertiary amine.

58. (Withdrawn) A dye compound as claimed in Claim 55, wherein R²⁰ is a linker element having the structure:

R²¹ is H or alkyl or aryl optionally substituted with one or more of SO₃-, PO₃²-, OH, O-alkyl, SH, S-alkyl, COOH, COO-, ester, amide, halogen, SO-alkyl, SO₂N-dialkyl, CN, secondary amine or tertiary amine and R²² is alkylene, O-alkylene, S-alkylene or N-alkylene or R²¹ and R²² are part of a ring, optionally substituted with one or more of SO₃-, PO₃²-, OH, O-alkyl, SH, S-alkyl, COOH, COO-, ester, amide, halogen, SO-alkyl, SO₂NH₂, SO₂NH-alkyl, SO₂N-dialkyl, SO₃-alkyl, CN, secondary amine or tertiary amine; and

 R^{23} is o-succinimidyl, o-pentafluorophenyl, Cl or α -halogenated alkyl.

- 59. (Withdrawn) A dye compound as claimed in Claim 55, wherein R¹⁰ to R¹⁵ are each Omethyl or O-ethyl.
- 60. (Withdrawn) A dye compound as claimed in Claim 55, further comprising one or more counterions selected from halide, BF₄, PF₆, NO₃, carboxylate, ClO₄, Li⁺, Na⁺, K⁺, Mg²⁺ and Zn²⁺.

NORRILD ET AL.

Appl. No. 10/582,794
January 25, 2010

61. (Withdrawn) A method of detecting or measuring an analyte using a reagent as claimed in Claim 33, comprising the steps of:

contacting the reagent with a sample;

illuminating the reagent and sample with light of wavelength within the absorption spectrum of the energy donor;

detecting non-radiative energy transfer between the energy donor and energy acceptor by measuring the fluorescence of the energy donor; and

associating the fluorescence measurements with presence or concentration of analyte.

- 62. (Withdrawn) A method as claimed in Claim 61, wherein the fluorescence of the energy donor is measured by measuring making intensity based or time resolved fluorescence measurements.
- 63. (Withdrawn) A method as claimed in Claim 61, wherein the analyte is measured by comparing sample fluorescence measurements with fluorescence measurements made using known concentrations of analyte.
- 64. (Withdrawn) A complex of an analyte and a reagent for detecting the analyte wherein the reagent comprises a fluorescent energy donor and an energy acceptor, the energy donor and the energy acceptor being such that when they are sufficiently close to one another energy is non-radiatively transferred from the energy donor following excitation thereof to the energy acceptor quenching fluorescence of the energy donor, wherein the energy acceptor is the compound of claim 33; and

wherein the presence of the analyte modulates the distance between the energy donor and the energy acceptor.

65. (New) A reagent as claimed in claim 33, in which:

NORRILD ET AL. Appl. No. 10/582,794 January 25, 2010

R¹ and R² are both NMe₂ or both MeNCH₂CH₂SO₃⁻

R³ is a linker element which is a succinimidyl ester

R⁴⁻⁹ are all H

R¹⁰⁻¹⁵ are all OMe;

the energy donor and energy acceptor are linked together by non-covalent binding which exists between an analyte binding agent linked to one of the energy donor and the energy acceptor and an analyte analogue linked to the other of the energy donor and the energy acceptor, the non-covalent binding being disruptable by the analyte so as to increase the distance between the energy donor and the energy acceptor of the reagent.

- 66. (New) A reagent as claimed in claim 65, wherein the analyte is glucose.
- 67. (New) A reagent as claimed in claim 66, wherein the analyte analogue is dextran.
- 68. (New) A reagent as claimed in claim 66, wherein the analyte binding agent is a lectin.
- 69. (New) A reagent as claimed in claim 65, wherein the energy donor is a dye that absorbs at 594 nm and fluoresces at 620 nm.